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ORIGINAL ARTICLE

Impact of the introduction of EUCAST's concept of "area of technical uncertainty"

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Abstract

On the first of January 2019, the European Committee on Antimicrobial Susceptibility Testing, EUCAST, introduced the concept of "area of technical uncertainty" (ATU). The aim was to report on the incidence of ATU test results in a selection of common bacterial species and the subsequent impact on antimicrobial resistance categorization and workload. A retrospective analysis of clinical samples collected from February 2019 until November 2019 was performed. Susceptibility to amoxicillin-clavulanic acid and piperacillin-tazobactam in *Enterobacterales* (*Escherichia* spp., *Klebsiella* spp., *Proteus* spp.), piperacillin-tazobactam in *Pseudomonas aeruginosa*, and amoxicillin-clavulanic acid and cefuroxime in *Haemophilus influenzae* was studied. Disk diffusion antibiotic susceptibility testing was read and interpreted by ADAGIO 93400 automated system (Bio-Rad, France). In case of an inhibition zone in the ATU, strains were retested using gradient minimal inhibitory concentration method (Etest, BioMérieux, France). Overall, 14,164 isolate-antibiotic combinations were tested in 7922 isolates, resulting in 1204 (8.5%) disk zone diameters in the ATU region. Retesting of ATUs with Etest resulted in a category change from S to R for amoxicillin-clavulanic acid in 63/498 (12.7%) of *Escherichia* spp., 2/58 (3.4%) of *Klebsiella* spp., 2/37 (5.4%) of *Proteus* spp., and 6/125 (4.8%) of *Haemophilus influenzae*. For piperacillin-tazobactam, a category change from S to R was found in 33/92 (35.9%) of *Pseudomonas aeruginosa*. We conclude that ATU testing has a substantial impact on the correct interpretation of antimicrobial resistance, at the expense of turn-around time and with the cost of additional workload.

Keywords ATU zone · Disk diffusion · Breakpoints · Antimicrobial resistance

Introduction

On the first of January 2019, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) introduced the new concept of "area of technical uncertainty" (ATU) and changed the definition of the "Intermediate" susceptibility category. Previously, the antibiotic susceptibility testing (AST) results were categorized into three groups: susceptible (S), intermediate (I), or resistant (R). The definition of susceptible and resistant was clear: "S" had a high likelihood of therapeutic success whereas "R" had a high likelihood of therapeutic failure. The category "I," until January 2019, left room for a broad interpretation. A microorganism was defined as "I" in case of antimicrobial agent activity associated with uncertain therapeutic effect. It implies that an infection due to the isolate may be appropriately treated in body sites where the drug is physiologically concentrated or when a high dosage of drug is administered. Besides, it indicated a buffer zone that should prevent (pre-)analytical variability from causing major discrepancies in interpretations [1] [2].

Given this ambiguous definition, clinicians might be reluctant to prescribe antibiotic agents reported as "I" and prefer an alternative antibiotic to which the isolate is reported sensitive. In times when multidrug-resistant organisms are a big health problem and the antibiotic options are limited, there was a need to clarify the definition. In June 2018, the decision was taken by EUCAST to update the definition of "I," meaning there is a high likelihood of therapeutic success when exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection [2]. To cover the (pre-)analytical variability, the new concept of "area of technical uncertainty" (ATU)

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was introduced. Application of EUCAST criteria alerts the laboratory staff that the value measured (mean inhibitory concentration (MIC) or inhibition zone) is situated in an area where the categorical interpretation (S, I, or R) may vary due to technical or methodological variation [2].

The objective of this study was to discuss the technical consequences associated with the application of the ATU in our laboratory setting and to measure its impact on antimicrobial resistance surveillance.

Material and methods

Bacterial isolates

From February to November 2019, a total of 7922 isolates were collected from various clinical specimens of patients who were hospitalized at the University Hospital of Ghent, Belgium, a tertiary care hospital. For these isolates, retrospective analysis of antimicrobial susceptibility results was performed. To this end, duplicate strains, i.e., the same sample type from the same patient on the same day, were excluded.

The isolates comprised a selection of micro-organisms for which EUCAST has defined an ATU (see Table 1).

The ATUs included in this study were amoxicillin-clavulanic acid (AMC) and piperacillin-tazobactam (TZP) for *Enterobacterales* (*Escherichia* species, *Klebsiella* species, and *Proteus* species), TZP for *Pseudomonas aeruginosa*, and AMC and cefuroxime (CXM) for *Haemophilus influenzae*.

Antimicrobial susceptibility testing

In our lab, disk diffusion (EUCAST standardized disk diffusion method) with paper disks (Biorad, France) was the preferred method for routine antibiotic susceptibility testing. For each strain, a 0.5 McFarland (McF) standard was prepared and applied to a Mueller-Hinton (MH) agar (+5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) for *Haemophilus influenzae*) and antibiotic disks were applied. After overnight incubation (16–20 h) at 35 °C (+5% CO_2 for *Haemophilus influenzae*), the ADAGIO 93400 automated system (Bio-Rad) was used for reading and interpreting the disk diffusion tests.

If the disk diffusion susceptibility test resulted in a zone diameter within the ATU, the result was yet not reported to the clinicians and instead a gradient minimal inhibitory concentration method (Etest, BioMérieux) was performed. As for the disk diffusion method, a 0.5 McF bacterial suspension was prepared and applied to a MH agar (MHF agar for *Haemophilus influenzae*), after which the Etest was applied. The minimum inhibitory concentration (MIC) results were read manually according to the manufacturer's protocol. MIC results were available for clinical use 24h after the disk diffusion results.

Data analysis

The disk diffusion zone diameters were grouped into susceptible, standard dosing regimen (S), susceptible, increased exposure (I), resistant (R) or ATU conform EUCAST Clinical Breakpoint Tables (v. 9.0, valid from 2019-01-01) [3]. Disk diameters within the ATU were detected with the ADAGIO software using an IF/THEN query, i.e., if the disk diffusion susceptibility test resulted in a zone diameter within the ATU, then the result for that specific antibiotic test was not sent to the LIS-system (Glims 9, MIPS) and, as such, not reported to the clinicians until a subsequent Etest was performed. Based on the MIC results, the strains were reclassified as S, R, or I and finally reported to the clinicians. Strains with MIC results that were again in the ATU were reported based on the EUCAST breakpoints (MIC), regardless of the ATU.

Table 1 ATU of disk diffusion zone and MIC for the included specimens. Adapted from EUCAST Breakpoint Table v 9.0, 2019

Species	Agent	MIC value (mg/L)			Zone diameters (mm)		
		S	R	ATU	S	R	ATU
Escherichia spp.	AMC	≤8	>8	/	≥19	<19	19-20
	TZP	≤8	>16	16	≥20	<17	17-19
Klebsiella spp.	AMC	≤8	>8	/	≥19	<19	19-20
	TZP	≤8	>16	16	≥20	<17	17-19
Proteus spp.	AMC	≤8	>8	/	≥19	<19	19-20
	TZP	≤8	>16	16	≥20	<17	17-19
P. aeruginosa	TZP	≤16	>16	/	≥18	<18	18-19
H. influenzae	AMC	≤2	>2	/	≥15	<15	14-16
	CXM	≤1	>2	2	≥27	<25	25-27

Abbreviations: *spp.*, species; *P. aeruginosa*, *Pseudomonas aeruginosa*; *H. influenzae*, *Haemophilus influenzae*; AMC, amoxicillin-clavulanic acid; *TZP*, piperacillin-tazobactam; *CXM*, cefuroxime

Results

Overall, 7922 clinical specimens were analyzed: *Escherichia* species (*n*=3502), *Klebsiella* species (*n*=1384), *Proteus* species (*n*=765), *Pseudomonas aeruginosa* (*n*=1680), and *Haemophilus influenzae* (*n*=591) (see Table 2).

From a total of 14,164 isolate-antibiotic combinations, 1204 (8.5%) resulted in zone diameters in the ATU region: 498/3502 (14.2%) for AMC and 151/3502 (4.3%) for TZP for *Escherichia* spp., 58/1384 (4.2%) for AMC and 126/1384 (9.1%) for TZP for *Klebsiella* spp., 37/765 (4.8%) for AMC and 5/765 (0.7%) for TZP for *Proteus* spp., 92/1680 (5.5%) for TZP for *P. aeruginosa* and 125/591 (21.2%) for AMC and 112/591 (19.0%) for CXM for *H. influenzae*.

Subsequent Etest resulted again in the ATU zone in 48/151 (31.8%) isolates for TZP in *Escherichia* spp. (MIC=16), 25/126 (19.8%) isolates for TZP in *Klebsiella* spp. (MIC=16), 1/5 isolates for TZP in *Proteus* spp. (MIC=16), and 7/112 (6.3%) isolates for CXM in *H. influenzae* (MIC=2).

A category change from S to R was observed in 63/498 (12.7%) isolates for AMC in *Escherichia* spp., 2/58 (3.4%) isolates for AMC in *Klebsiella* spp., 2/37 (5.4%) isolates for AMC in *Proteus* spp., 33/92 (35.9%) isolates for TZP in *Pseudomonas aeruginosa*, 6/125 (4.8%) isolates for AMC, and 4/112 (3.6%) isolates for CXM in *H. influenzae*. A category change from R to S was observed in 12/125 (9.6%) isolates for AMC in *Haemophilus influenzae*.

Category changes from I to R were seen in 42/151 (27.8%) isolates for TZP in *Escherichia* spp., 19/126 (15.1%) isolates for TZP in *Klebsiella* spp., 1/5 isolates for TZP in *Proteus* spp., and 2/112 (1.8%) isolates for CXM in *Haemophilus influenzae*.

Category changes from I to S were seen in 29/151 (19.1%) isolates for TZP in *Escherichia* spp., 55/126 (43.7%) isolates for TZP in *Klebsiella* spp., and 35/112 (31.3%) isolates for CXM in *Haemophilus influenzae*.

Discussion

On the first of February 2019, the microbiology lab of the Ghent University Hospital implemented the new concept of ATU, as proposed by the EUCAST. The renewed definition and interpretation of the category "I" was approved by the local antibiotic Policy Coordination Committee and all clinicians were updated by means of a newsletter.

In this study, the impact of the introduction of ATU, in routine practice, on the antimicrobial resistance surveillance and on the laboratory workflow was studied.

During the study period, 14,164 isolate-antibiotic combinations were tested of which an important share (8.5%) of the disk zone diameters resulted in the ATU region. EUCAST advises to perform an alternative test method for breakpoints resulting in the ATU zone. In this study, we performed an Etest as the alternative test method.

With the Etest, 314/1204 (26.1%) strains in the ATU zone resulted in a category change (=inconclusive result). EUCAST indicates that the result of this alternative test method is only relevant if the results of both tests are conclusive. If the test results are inconclusive, EUCAST suggests to report the result in ATU as uncertain or as resistant if there are enough alternative antibiotic treatment options available. In contrast to the suggestion of EUCAST, we valued the outcome of the Etest as the final antibiotic susceptibility result, both in conclusive and inconclusive situations.

As such, the impact on the interpretation of antimicrobial resistance was high for some specific isolate-antibiotic combinations with category change from S to R for TZP in 35.9% of *Pseudomonas aeruginosa*, and for AMC in 12.7% of *Escherichia* spp.

So far, literature on the impact of the ATU on the antimicrobial resistance pattern and the impact on the lab organization is limited. In our study, 4.3% (151/3502) of zone diameter obtained for *Escherichia* spp. resulted in an ATU zone for TZP. This is in agreement with the 3–4% mentioned by EUCAST based on 6033 observations [2]. Furthermore, Soares et al. analyzed the area of technical uncertainty for susceptibility testing of AMC against *Escherichia coli* urinary strains using three different methods: automated Phoenix system (Becton Dickinson, France), disk diffusion (Bio-Rad, France), and Etest (AES, France), with broth microdilution in 96-well microtiter plates as gold standard. They confirmed the 19–20-mm ATU for the disk diffusion method and suggested introducing an ATU for Etest AMC MIC values of 6 and 8 mg/L [4]. In our cohort of *Escherichia* spp., 154/498 had a E-test MIC result of 6 mg/L and 252/498 had a E-test MIC result of 8 mg/L.

Ballestero-Tellez et al. evaluated the accuracy of various susceptibility testing methods for CXM against *E. coli* with CXM MIC values of 16 mg/L, as analyzed by broth microdilution (Vitek 2). The strains were tested by reference standard microdilution, disk diffusion (using Oxoid and Bio-Rad disks) and MIC gradient tests (using BioMérieux and Liofilchem). They concluded that the inter-technique variation around the CXM breakpoint of 16 mg/dL had a great impact on the susceptibility classification [5]. This is in line with our findings that the results of the disk diffusion and Etest are frequently inconclusive and emphasize the technical issues of antibiotic susceptibility testing and the importance of the ATU.

Besides the impact on the antibiotic susceptibility, additional testing of isolate-antibiotic combinations in the ATU

Table 2 Results of susceptibility testing and category changes after retesting results in ATU region

Species	Antimicrobial agent	No. *susceptible* results (disk diffusion)	No. *resistant* results (disk diffusion)	No. of results in ATU (disk diffusion)			Total isolates in ATU region (%)	*Clinical impact of ATU definition and additional E-test*				
				No. *susceptible* results (E-test)	No. *intermediate* results (E-test)	No. *resistant* results (E-test)		*Category change S to R*	Category change R to S	*Category change I to R*	Category change I to S	Category change S to I
Escherichia spp. (3502 isolates)	**AMC**	2300	704	435	-	63	*498/3502 (14.2%)*	*63/498 (12.7%)*	-	-	-	-
	TZP	3101	250	29	80	42	*151/3502 (4.3%)*	-	-	*42/151 (27.8%)*	*29/151 (19.1%)*	-
Klebsiella spp. (1384 isolates)	**AMC**	989	337	56	-	2	*58/1384 (4.2%)*	*2/58 (3.4%)*	-	-	-	-
	TZP	1012	246	55	52	19	*126/1384 (9.1%)*	-	-	*19/126 (15.1%)*	*55/126 (43.7%)*	-
Proteus spp. (765 isolates)	**AMC**	695	33	35	-	2	*37/765 (4.8%)*	*2/37 (5.4%)*	-	-	-	-
	TZP	757	3		4	1	*5/765 (0.7%)*	-	-	*1/5 (20.0%)*	-	-
Pseudomonas aeroginosa (1680 isolates)	**TZP**	1248	340	59	-	33	*92/1680 (5.5%)*	*33/92 (35.9%)*	-	-	-	-
Haemophilus influenzae (591 isolates)	**AMC**	379	87	117	-	8	*125/591 (21.2%)*	*6/125 (4.8%)*	*12/125 (9.6%)*	-	-	-
	CXM	304	175	92	14	6	*112/591 (19.0%)*	*4/112 (3.6%)*		*2/112 (1.8%)*	*35/112 (31.3%)*	*9/112 (8.0%)*
Total of 7922 isolates		10785	2175	878	150	176	*1204/14164 (8.5%)*	*110*	*12*	*64*	*119*	*9*

Abbreviations: *ATU*, area of technical uncertainty; *No.*, number; *spp.*, species; *AMC*, amoxicillin-clavulanic acid; *TZP*, piperacillin-tazobactam

implies a delay in reporting the result to the clinician. Furthermore, extra testing involves supplementary laboratory costs including an increased work load.

As study limitation, we acknowledge that our results were not compared to the reference method which is broth microdilution. Secondly, due to cost and time constraints, the disk diffusion and Etest were not tested in duplicate.

Importantly, we did not follow the suggestion of EUCAST to not report the inconclusive antibiotic susceptibility test results and leave it blank with comment "unreliable susceptibility test result" or downgrade the susceptibility category (S to R). Instead, we reported the outcome of the Etest as the final result available for the clinicians. It was our opinion that not reporting the inconclusive test results or downgrading the susceptibility category would limit the choice of currently used antibiotic treatment options, i.e., AMC or TZP. Especially since, almost 10% of the included strains resulted in the ATU zone of which one out of four (314/1204) could not be confirmed by Etest. Ideally, the laboratory information system (GLIMS 9.0, MIPS, Belgium) should support automatic notes in the report so that clinicians can be warned when some antibiotic susceptibility results are within the ATU. Fortunately, our hospital has a long-standing history of multidisciplinary meetings as well as brief communications between clinicians and microbiologists that allow discussion of these susceptibility issues in challenging infection cases and searching for suitable patient-specific antibiotic treatment.

We can conclude that an important percentage of isolate-antibiotic combinations resulted in a disk zone diameter in the ATU zone. Additional testing resulted in up to 26% inconclusive results which confirms the technical uncertainties presented by EUCAST.

Additional testing due to ATU implementation has a substantial impact on the laboratory workflow and led to clinically relevant antibiotic susceptibility changes, in particular for TZP: a category change from S to R was observed in 35.9% of *Pseudomonas aeruginosa* and 12.7% in *Escherichia* spp.

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Data availability Not applicable

Code availability Not applicable

Declarations

Ethics approval Not required

Consent to participate Not applicable

Consent for publication All the authors agree for publication of this manuscript.

Conflict of interest The authors declare no competing interests.

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